Conformation and dynamics of the deoxyribose rings of a (nogalamycin)₂-d(5'-GCATGC)₂ complex studied in solution by ¹H-n.m.r. spectroscopy

Mark S. SEARLE and Laurence P. WAKELIN

Molecular Pharmacology Group, Peter MacCallum Cancer Institute, 481 Little Lonsdale St., Melbourne, Victoria 3000, Australia

The conformation and dynamics of the deoxyribose rings of a (nogalamycin)₂-d(5'-GCATGC)₂ complex have been determined from an analysis of ¹H-¹H vicinal coupling constants and sums of coupling constants ($J_{1'-2'}$, $J_{1'-2''}$, $\Sigma 1'$, $\Sigma 2'$ and $\Sigma 2''$) measured from one-dimensional n.m.r. spectra and from H-1'-H-2' and H-1'-H-2'' cross-peaks in high-resolution phase-sensitive two-dimensional correlation spectroscopy (COSY) and double-quantum-filtered correlation spectroscopy (DQF-COSY) experiments. The value of $J_{3'-4'}$ has also been estimated from the magnitude of H-3'-H-4' cross-peaks in DQF-COSY spectra and H-1'-H-4' coherence transfer cross-peaks in two-dimensional homonuclear Hartman-Hahn spectroscopy (HOHAHA) spectra. The data were analysed, in terms of a dynamic equilibrium between North (C-3'-endo) and South (C-2'-endo) conformers, by using the graphical-analysis methods described by Rinkel & Altana [(1987) J. Biomol. Struct. Dyn. 4, 621–649]. The data reveal that the sugars of the 2C-5G and 3A-4T base-pairs, which form the drug-intercalation site, have strikingly different properties. The deoxyribose rings of the 2C-5G base-pair are best described in terms of an equilibrium heavily weighted in favour of the C-2'-endo geometry (> 95% 'S'), with a phase angle, P , lying in the range 170–175° and amplitude of pucker between 35 and 40°, as typically found for B-DNA. For the deoxyribose rings of the 3A-4T base-pair, however, the analysis shows that, for 3A, the C-2'-endo and C3'-endo conformers are equally populated, whereas a more limited data set for the 4T nucleotide restricts the equilibrium to within 65–75% C-2'-endo. The deoxyribose rings of the 1G-6C base-pair have populations of 70–80% C-2'-endo, typical of nucleotides at the ends of a duplex. Although drug-base-pair stacking interactions are an important determinant of the enhanced duplex stability of the complex [Searle, Hall, Denny, & Wakelin (1988) Biochemistry 27, 4340–4349], the current findings make it clear that the same interactions can be associated with considerable variations in the degree of local structural dynamics at the level of the sugar puckers.

INTRODUCTION

Nogalamycin (Fig. 1) is a naturally occurring anthracycline antibiotic, active against Gram-positive bacteria and experimental tumours [1–3], whose intercalative binding to DNA has been well studied by a variety of physical techniques [4–7]. Recently we described a low-resolution three-dimensional structure of a two-fold symmetric (nogalamycin)₂-d(GCATGC)₂ complex [8] based upon a detailed analysis of two-dimensional (2D) nuclear-Overhauser-effect (n.O.e.) data. Our findings showed that drug molecules were bound at each of the equivalent 5'-CpA and 5'-TpG high-affinity binding sites and provided some explanation for the specificity that nogalamycin shows for the 5'-GCA (5'-TGC) trinucleotide sequence previously identified in footprinting studies [9]. The major determinants of specificity appeared to be hydrogen-bonding and electrostatic interactions in the major groove, principally between the O-6 and N-7 atoms of the guanine base at the intercalation site and the hydroxy groups and charged *NN*-dimethylamino group of the bicyclic sugar of the antibiotic. The O-6 of the terminal guanine is also suitably positioned to interact with these same functional groups on the antibiotic, so extending the binding site to a trinucleotide sequence. In this earlier work we characterized the deoxyribose ring conformations in terms of the broad envelope of 'C-2'-endo' geometries, based upon a consideration of both the pattern and intensities of cross-peaks identified in NOESY (2D n.O.e.

spectroscopy) spectra [8]. It is now appropriate to present a higher-resolution study of the structure and dynamics of the backbone of the oligonucleotide duplex based upon a quantitative analysis of deoxyribose ¹H-¹H coupling constants derived from one-dimensional (1D) and high-resolution 2D n.m.r. data. This approach has proved to be more reliable for detecting subtle variations in deoxyribose conformation and dynamics than has

Fig. 1. Chemical structure of nogalamycin

Abbreviations used: 1D and 2D, one- and two-dimensional; COSY, 2D correlation spectroscopy; DQF-COSY, 2D double-quantum-filtered correlation spectroscopy; NOESY, 2D nuclear-Overhauser-effect spectroscopy; n.O.e., nuclear Overhauser effect; HOHAHA, 2D homonuclear Hartman-Hahn spectroscopy; RMS, root mean square; P , pseudo-rotation phase angle.

* To whom correspondence and reprint requests should be sent.

the use of interproton distance information derived from n.O.e. data [10–12]. The findings have been examined in terms of a conformational equilibrium between North (C-3'-endo) and South (C-2'-endo) geometries, using the graphical analysis approach described by Rinkel & Altona [13]. The data reveal that nogalamycin has an unusual and contrasting effect on the conformation and dynamics of the deoxyribose rings of the G–C and A–T base-pairs forming the drug-binding site. Dynamic properties identified in these solution studies are not apparent in complexes of this and other anthracycline antibiotics observed in the crystalline state [14–16].

MATERIALS AND METHODS

The complex used in these studies is the same as that previously described [8]. N.m.r. measurements were made at 400 MHz on a Varian VXR400 W/B spectrometer, and the data were processed on a Sun 3/50 data station. Pure absorption phase-sensitive 2D correlation spectroscopy (COSY), 2D double-quantum-filtered COSY (DQF-COSY) and 2D homonuclear Hartman–Hahn spectroscopy (HOHAHA) spectra were obtained using the hypercomplex method of data collection with the carrier frequency placed at the centre of the spectrum [17,18]. A spectral width of 3600 Hz was employed with relaxation delays of 1.2–1.6 s incorporated into each sequence. COSY and DQF-COSY spectra were acquired as 2048 complex points in t_2 for 2×512 t_1 increments zero-filled to a 4096×2048 matrix prior to Fourier transformation using shifted-sinebell apodization functions. Digital resolution was 0.88 Hz/point in ω_2 and 1.76 Hz/point in ω_1 . HOHAHA spectra with isotropic mixing periods of 43, 69, 110, 148 and 250 ms were acquired as 1024 points in t_2 for 2×300 –400 t_1 increments and zero-filled to 1024 points in t_1 before transformation. In this case mild squared-Gaussian window functions were used to weight the data. Nucleotides are

numbered sequentially from the 5'-end of the hexamer, namely d(1G2C3A4T5G6C). Resonance assignments and ^1H chemical shifts for both the free and complexed hexamer were reported previously [8].

RESULTS AND DISCUSSION

Determination of coupling constants

The deoxyribose ring gives rise to five vicinal scalar coupling constants, many of which show large conformation-dependent variations in magnitude. Several approaches have been described for extracting individual coupling constants from complex spectra. These include, for example, the application of pulse sequences, which increase multiplet resolution through ω_1 -scaling effects [19] or lead to simplified 2D cross-peaks by selecting out only those directly-connected transitions that contribute to the multiplet structure (Exclusive COSY, Primitive Exclusive COSY [20–22]). The graphical analysis methods proposed by Rinkel & Altona [13], which are based on easily determined coupling constants extracted from conventional phase-sensitive COSY spectra, have found widespread application to the study of oligonucleotides in solution. Here direct measurement of coupling constants ($J_{1'-2'}$ and $J_{1'-2''}$) from well-resolved regions of coupling constants ($J_{1'-2'}$ and $J_{1'-2''}$) from well-resolved regions of resolution-enhanced 1D n.m.r. spectra has been combined with measurements of the sums of several coupling constants ($\Sigma 1' = J_{1'-2'} + J_{1'-2''}$, $\Sigma 2' = J_{1'-2'} + J_{2'-3'} + J_{2'-3''}$, and $\Sigma 2'' = J_{1'-2''} + J_{2'-3'} + J_{2'-3''}$) derived from the overall width of cross-peak multiplets observed in high-resolution phase-sensitive COSY spectra. The relationship between the magnitude of the active coupling and the relative intensity of cross-peaks in both DQF-COSY and isotropic mixing experiments (HOHAHA) have also enabled a semi-quantitative estimate to be made for the value of $J_{3'-4'}$. This combination of methods proved successful in a

Fig. 2. Contour plots of the H-1'–H-2' and H-1'–H-2'' cross-peaks from the phase-sensitive COSY spectrum of the (nogalamycin)₂–d(GCATGC)₂ complex, recorded at 25 °C, and the corresponding H-1' resonances from the resolution-enhanced 1D n.m.r. spectrum

Assignments are indicated at the top of each column, and chemical shifts are as previously reported [8]. For 4T, the individual cross-peaks are not resolved, and the contour plot represents the amalgamated peak. In all cases positive and negative contour levels are plotted without distinction.

Fig. 3. Contour plot of the H-3'-H-4' cross-peak region of the phase-sensitive DQF-COSY spectrum of the (nogalamycin)₂-d(GCATGC)₂ complex at 25 °C

Assignments are indicated for each sugar residue, with circles representing the positions of weak or unobserved cross-peaks.

situation where direct measurement of a large number of individual coupling constants from 1D spectra is limited by poor spectral dispersion and, in the case of 2D spectra, where overlap of phased components lead to cancellation and amalgamation effects within the cross-peak multiplet structure.

The 1D n.m.r. spectrum of the (nogalamycin)₂-d(GCATGC)₂ complex offers sufficient resolution of only the sugar H1' resonances (5.5–6.20 p.p.m.), whereas H-2'/H-2'' (1.80–2.90 p.p.m.) and H-3' (4.50–5.00 p.p.m.) suffer severely from resonance overlap, and no useful coupling information can be obtained directly from the H2', H2'' and H3' signals. In resolution-enhanced 1D spectra the splitting patterns of the six H1' resonances (Fig. 2) reveal considerable variation in the

relative magnitude of $J_{1'-2'}$ and $J_{1'-2''}$. In cases where $|J_{1'-2'} - J_{1'-2''}|$ is small (< 2 Hz) the H-1' resonance appears as a triplet (Fig. 2), with the frequency difference between the outer components of the multiplet representing $\Sigma 1'$. Considerable variation in the value of $\Sigma 1'$ is also evident from the outer splittings of H-1'-H-2' and H-1'-H-2'' cross-peaks observed in phase-sensitive COSY spectra, as illustrated in Fig. 2. The cross-peaks of 3A and 5G represent the extreme examples. Further distinctions from both the splitting pattern and the magnitude of $\Sigma 2'$ and $\Sigma 2''$ are readily identified when the multiplet structure is examined along ω_2 . The H1'-H2' cross-peaks are resolved into intense outer components which, in the case of 1G, 2C and 5G, have a partially resolved in-phase splitting which is not apparent for the cross-peaks of 3A and 6C. For 4T the fine structure is less well resolved because of considerable overlap between the H-1'-H-2' and H-1'-H-2'' cross-peaks, making a detailed analysis difficult. The H-1'-H-2'' cross-peaks generally show less variation in fine structure and are readily resolved into four components along the ω_2 axis. Values for $\Sigma 1'$, $J_{1'-2'}$ and $J_{1'-2''}$ were measured directly from resolution-enhanced 1D spectra and, in the case of 3A and 6C, from the results of spin-decoupling experiments where H-2' and H-2'' resonances could be selectively irradiated (results not shown). The sums of coupling constants $\Sigma 2'$ and $\Sigma 2''$ were extracted from phase-sensitive COSY data, as illustrated in Fig. 2. With the exception of 4T, the ratio $\Delta\delta/J$ for H-2' and H-2'' proton pairs is larger than 1.5, which, according to the simulation studies by Rinkel & Altona [13], enables accurate first-order measurements to be made for individual coupling constants from the H-1' signals. In the case of the 4T sugar the chemical shift difference between H-2' and H-2'' is comparable with $J_{2'-2''}$ ($\Delta\delta/J \sim 1.0$), which leads to both poor resolution and significant distortion of the multiplet structure. A first-order analysis is only appropriate, therefore, for determining the value of $\Sigma 1'$, which is unaffected by $\Delta\delta$ for H-2' and H-2'' [13]. The final vicinal coupling constant used to define the sugar geometry, $J_{3'-4'}$, has been estimated from the magnitude of cross-peaks mediated by the H-3'-H-4' coupling. The portion of the phase-sensitive DQF-COSY spectrum containing H-3'-H-4' cross-peaks is illustrated in Fig. 3. Strong correlations are observed for 3A, 4T and the terminal 6C, whereas those for all other residues are

Fig. 4. Pure absorption phase-sensitive HOHAHA spectrum of the (nogalamycin)₂-d(GCATGC)₂ complex at 25 °C (isotropic mixing period, 148 ms) illustrating correlations from the deoxyribose H-1' resonances to H-2', H-2'' and H-3', in all cases

For 3A, 4T and 6C additional H-1'-H-4' correlations are also observed, and these are highlighted in boxes.

Table 1. ^1H – ^1H coupling constants (in Hz), sums of coupling constants (in Hz) and conformation (phase angle, P , and pucker amplitude, Φ) of the deoxyribose rings of the (nogalamycin) $_2$ –d(GCATGC) $_2$ complex at 25 °C

Coupling constants from 1D spectra are ± 0.2 Hz; sums of coupling constants from 2D data are ± 0.88 Hz. $J_{3'-4'}$ is assigned a value of weak (w) or strong (s) on the basis of an analysis of 2D data as described in the text. Values in parentheses are coupling constants calculated using the two-state model and the data on the limiting structures described by Rinkel & Altona [13]. The two-state model describes the percentage of C-2'-endo ring pucker, % 'S', with phase angle, P_s , and pucker amplitude, Φ , in dynamic equilibrium with the C-3'-endo conformer, $P = 9^\circ$ and $\Phi = 40^\circ$. RMS is the root-mean-square difference between the experimental coupling constants and the calculated values.

Base	$J_{1'-2'}$	$J_{1'-2''}$	$\Sigma 1'$	$\Sigma 2'$	$\Sigma 2''$	$J_{3'-4'}$	% 'S'	P_s	Φ	RMS
1G	8.2 (8.2)	5.8 (5.8)	14.0 (14.0)	28.3 (27.3)	22.1 (23.1)	w	80	180	40	0.11
2C	9.8 (9.8)	5.6 (5.7)	15.4 (15.5)	28.9 (29.4)	20.6 (21.0)	w	> 95	171	35	0.02
3A	5.7 (5.8)	6.7 (6.7)	12.4 (12.5)	26.9 (26.5)	26.0 (26.2)	s	50	162	35	0.06
4T	—	—	14.2	—	—	s	65–75	128–189	35	—
5G	10.0 (10.1)	5.2 (5.2)	15.2 (15.3)	29.2 (28.8)	20.8 (20.8)	w	< 95	175	40	0.11
6C	7.9 (7.9)	6.3 (6.4)	14.2 (14.2)	28.9 (29.4)	23.4 (23.9)	s	70	126	35	0.28

barely visible above the noise level. A further manifestation of the apparently larger value of $J_{3'-4'}$ for 3A, 4T and 6C is observed in isotropic mixing experiments. In the HOHAHA spectrum of Fig. 4, recorded with a spin-locking period of 148 ms, long-range H-1'–H-4' correlations are observed only for 3A, 4T and the terminal residue 6C, confirming that $J_{3'-4'}$ is moderately large for these residues, but small for 1G, 2C and 5G. In HOHAHA spectra with even longer mixing periods (250 ms; results not shown) total correlation from H-1' through to H-5' is observed for 3A, 4T and 6C, whereas magnetization is propagated no further than H-3' in the case of 1G, 2C and 5G. Thus a study of the time-dependence of coherence transfer in isotropic mixing experiments provides a semi-quantitative approach to extracting J -coupling information in poorly resolved spin systems. The coupling-constant data are summarized in Table 1.

Deoxyribose ring conformation and dynamics

The two lowest-energy conformers, C-3'-endo (pseudo-rotation phase angle, P , = 9°) and C-2'-endo (P = 162°), give rise to quite different values for $J_{1'-2'}$, $J_{1'-2''}$, $\Sigma 1'$, $\Sigma 2'$ and $\Sigma 2''$ and provide an initial handle on the predominant conformation of the deoxyribose ring in solution. When $\Sigma 1' > 13.5$ Hz, $\Sigma 2' > 28.0$ Hz and $\Sigma 2'' < 23.0$ Hz the predominant conformation is broadly identified in terms of the C-2'-endo geometry. In the fast-dynamic-equilibrium model (rate of interconversion is $\gg J$) a phase angle of 9° and a pucker amplitude of 40° were assumed for the minor (C-3'-endo) conformer. The position of the equilibrium, phase angle and pucker amplitude of the major conformer were determined by using the graphical analysis approach described by Rinkel & Altona [13]. The coupling constants observed for 2C and 5G are typical of a geometry close to the classical C-2'-endo 'B-DNA' conformation, with the North–South equilibrium lying strongly in favour of the 'S' conformer (> 95%; see Table 1). In both cases minimum root-mean-square (RMS) differences (< 0.12 Hz) between experimental and calculated couplings were obtained for phase angles in the range 170 – 175° and amplitudes of pucker between 35 and 40° . For 1G, however, the value of $J_{1'-2'}$ is 1.6–1.8 Hz smaller than for 2C and 5G, whereas the value of $J_{1'-2''}$ is marginally higher. The data could only partially be explained by invoking a single conformer with P in the range 195 – 205° . A more satisfactory solution was obtained by considering an equilibrium involving 80% of a conformer centred on $P = 180^\circ$ and Φ (pucker amplitude) = 40° (see Table 1). For 3A, the abnormally small value of $\Sigma 1'$ and the large value of both $\Sigma 2''$ and $J_{3'-4'}$ again identify the effects of conformational averaging, but indicate that a much greater contribution of the 'N'-type geometry is involved in the equilibrium than is observed for the other nucleotide sugars. The data are best rationalized in

terms of an equilibrium between a C-2'-endo conformer ($P = 162^\circ$, $\Phi = 35^\circ$) and 50% of the C-3'-endo geometry. The data for 6C similarly indicate conformational heterogeneity of the deoxyribose ring to the extent of a 30% contribution from the C-3'-endo geometry, with $P = 126^\circ$ for the major component of the equilibrium. The data set for 4T is incomplete, and the geometry correspondingly less well defined; however, estimates of the position of the conformational equilibrium from the values of $\Sigma 1'$ and $J_{3'-4'}$ alone suggest considerable dynamic distortions of the sugar geometry. The value of $\Sigma 1'$ is intermediate between the values observed for 3A and 5G, and this value alone restricts the conformation of 4T to an equilibrium involving 65–80% of a C-2'-endo geometry with possible values of P in the range 126 – 189° . The value of $J_{3'-4'}$ is also predicted to vary considerably over this range of equilibrium populations, and, given that $J_{3'-4'}$ for 4T appears to be moderately large, comparable with that observed for 3A but not for 1G, it is concluded that a narrower range of 65–75% C-2'-endo more adequately describes the data for 4T.

With the exception of the conformation of the deoxyribose ring of 4T, which is underdetermined by the experimental data, RMS differences between experimental and calculated coupling constants and sums of coupling constants were readily reduced to less than 0.3 Hz, and in most cases less than 0.12 Hz, by considering the two-state model. It is concluded that this model produces reasonable descriptions of the sugar rings of the (nogalamycin) $_2$ –d(GCATGC) $_2$ complex compared with the results of fitting the data to a single C-2'-endo conformer, where RMS values greater than 1.0 Hz were typically observed. The results are summarized in Table 1.

Overview of the structure and dynamics of the complex

The data presented establish the existence of wide-ranging conformational heterogeneity among the deoxyribose rings of the (nogalamycin) $_2$ –d(GCATGC) $_2$ complex and are summarized schematically in Fig. 5. It is found that the contribution to the conformational equilibrium from the 'N' conformer varies greatly, but that the phase and pucker amplitude of the 'S' component fall within the normally observed range of 126 – 189° and 35 – 40° respectively. The deoxyribose rings of 1G and 6C have populations of 70–80% C2'-endo, typical of nucleotides at the end of a duplex [10,18], whereas the deoxyribose rings of the 2C–5G base-pair at the drug-binding site have equilibria that are heavily weighted in favour of the 'S' geometry (< 95%). In marked contrast, considerable conformational heterogeneity is observed for the deoxyribose rings of the 3A–4T base-pairs, which also flank the bound drug molecules and constitute the core of the duplex. For 3A the analysis shows that the C-2'-endo and C-3'-endo conformers are equally populated, whereas the

Fig. 5. Schematic representation of the (nogalamycin)₂-d(GCATGC)₂ complex with drug molecules intercalated at the 5'-CpA and 5'-TpG steps

The percentage of the C-2'-endo conformer that contributes to the North (C-3'-endo)↔South (C-2'-endo) conformational equilibrium is indicated for each nucleotide.

limited data on 4T restricts the equilibrium in the range 65–75% C-2'-endo. Thus the pattern of 'high-purity' purine-sugar pucker that is generally observed for *B*-DNA in solution [13] does not emerge in this case. In fact, quite the contrary. The 'lowest-purity' sugar pucker in this data set belongs to the purine nucleotide 3A. Equally significant is the position of the equilibrium for this nucleotide, which falls well outside the normal range (> 70% C-2'-endo) observed for *B*-DNA [13]. Neither do any simple rules emerge to describe the conformation and dynamics of the deoxyribose rings at the 3'- or 5'-side of the intercalation site, since the pyrimidine and purine sugars of the 5'-CpA and 5'-TpG steps have quite different properties. In our previously reported study of this complex [8] it was found that nogalamycin binding appreciably stabilizes the duplex towards thermal denaturation and dramatically lowers the rate of exchange of the non-terminal G–C and A–T imino protons with the protons of the bulk solvent. These observations strongly implicate drug–base-pair stacking interactions as an important determinant of duplex stability and base-pair breathing motions, yet the current findings make it clear that these same interactions can be associated with considerable variations in the degree of local structural dynamics at the level of the sugar puckers.

Some comment on the structure and dynamics of d(GCATGC)₂ alone is appropriate at this point. The hexanucleotide duplex has been studied in detail by using time-dependent n.O.e. measurements and restrained molecular dynamics by Nilges *et al.* [23]. From their n.O.e. data they conclude that the sugar puckers lie in the O-1'-endo/C-2'-endo range, but make no use of coupling-constant data in their refinement and do not consider the possibility that the deoxyribose rings are involved in dynamic equilibria. Our own data (M. S. Searle & L. P. G. Wakelin, unpublished work) indicate that the conformation of the hexamer duplex is dominated by 'end-effects'. Very short base-pair lifetimes are observed, in contrast with those

observed in the complex. The splitting patterns for the well-resolved H-1' resonances and the values of $\Sigma 1'$ are indicative of dynamic equilibria in the range 75–85% C-2'-endo, typical of other short oligonucleotide duplexes [10]. However, the extreme values of 50% and > 95% C-2'-endo observed in the complex are not apparent for the hexamer duplex alone. Thus it can be concluded that the conformation and dynamics of the deoxyribose rings observed for the complexed hexamer are a consequence of nogalamycin binding.

Very few high-resolution data are available on deoxyribose conformation and dynamics from other drug–DNA complexes studied in solution; thus it is not possible to comment on general trends in sugar dynamics at intercalation sites. A number of theoretical energy-minimization studies have, however, addressed the question of the relationship between local helix twist-angle and backbone conformation in DNA. The results indicate that, in underwound helices, there is a pronounced tendency for sugars to repucker to the O-4'-endo or C-3'-endo configurations [24]. Taken to the extreme cases of insertion of planar ligands into the DNA framework, pronounced local unwinding of the DNA backbone is required. Evidence for this process from work in this laboratory is provided by the observation of marked downfield changes in ³¹P chemical shifts upon binding of nogalamycin to d(GCATGC)₂ [8]. Theoretical studies indicate that the majority of possible intercalated structures adopt C-3'-endo puckering in the 3'-sugar, whereas the 5'-sugar exhibits no conformational preference [25]. Most studies agree that the sugar–phosphate backbone of DNA helical structures is very flexible and gives rise to a large number of possible binding-site geometries. Taylor & Olson [25] conclude from their potential-energy studies that the occurrence of certain DNA conformations over others will ultimately be determined by ligand-specific interactions with the sugar–phosphate backbone. Experimental data on a number of related crystalline structures, studied at high-resolution, are also available for comparison with the present study. In the complexes of the anthracycline antibiotic daunomycin with d(CGTACG)₂ and d(CGATCG)₂ [14,15], rigid three-dimensional structures are reported. No simple rules concerning the conformation of the deoxyribose rings emerge, with all sugar geometries falling within the range $P = 104^\circ$ (O-4'-endo) and $P = 182^\circ$ (C-3'-exo), characteristic of *B*-DNA. Very recently the crystal structures of two nogalamycin molecules bound to the CpG sites of d[CGT(pS)ACG]₂ and d[m⁵CGT(pS)Am⁵CG]₂ (where pS is thiophosphate) have been reported [16]. The mode of binding of the antibiotic to these hexamers agrees closely with the structure proposed for (nogalamycin)₂-d(GCATGC)₂ from our n.m.r. data [8]. In these complexes the sugar puckers also fall in the C-2'-endo region, except for that of the terminal C1 nucleotide, which is C-3'-endo. Again, no obvious pattern of mixed sugar puckering is observed. Thus the solution studies yield dynamic information not apparent from studies of complexes in the crystalline state. Finally, investigators should consider the possibility and implications of pronounced conformational heterogeneity within the deoxyribose rings of drug–DNA complexes before embarking upon detailed structural studies involving a quantitative interpretation of n.O.e. data.

We are most grateful to Professor Bill Denny for providing the oligonucleotide used in these studies. This work was supported by the Australian Research Council.

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Received 5 December 1989/9 February 1990; accepted 14 February 1990